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Swelling-activated organic osmolyte efflux: A new role for anion channels

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Efflux of intracellular electrolytes and small organic solutes termed organic osmolytes is a universal response to cell swelling. While volume-activated ion efflux pathways have been studied extensively, relatively little was known until recently about the mechanisms and regulation of organic osmolyte release. An accumulating body of evidence, however, now indicates that structurally dissimilar organic osmolytes are lost from cells via a swelling-activated anion channel. We have termed this channel VSOAC for *v*olume-*s*ensitive *o*rganic osmolyte-*a*nion *c*hannel. The purpose of this review is to summarize our current understanding of VSOAC and the process of volume regulatory organic osmolyte efflux.

Organic osmolytes

Small organic molecules that serve as intracellular osmotic effectors are termed organic osmolytes. In mammalian cells these solutes fall into three broad classes: polyols (such as sorbitol, myo-inositol), amino acids and their derivatives (such as taurine, proline, alanine) and methylamines (such as betaine, glycerophosphorylcholine). Organic osmolytes are found in high concentrations (tens to hundreds of millimolar) in the cytosol of all organisms from bacteria to humans [1, 2]. These solutes play a central role in cellular osmoregulation [1–3] and may carry out specific 'cytoprotective' functions [4].

Intracellular organic osmolyte concentrations are elevated in response to increases in extracellular osmolality. The accumulation of these solutes is mediated primarily by energy-dependent transport or synthesis from metabolic precursors [2, 3]. Cell swelling leads to the loss of organic osmolytes from cells. This loss occurs largely by a very rapid (seconds), swelling-induced increase in passive organic osmolyte efflux to the external medium. Swelling-induced organic osmolyte efflux has been observed in most major groups of organisms including bacteria [5], algae [6], plants [7; R.A. Bressan, personal communication], invertebrates [8, 9], lower vertebrates [10–13] and mammals [14–20].

Characteristics of organic osmolyte efflux pathways

Volume-sensitive amino acid efflux mechanisms have been observed in a variety of cell types including skate [10, 21] and teleost [12, 13] erythrocytes, molluscan blood cells [9], crab axons [8], skate hepatocytes [11], renal epithelial cells [14, 15, 22], glial cells [16, 17, 23, 24], human lung cells [18], lymphocytes [19] and Ehrlich ascites cells [20]. The taurine efflux pathway has been characterized in detail by several laboratories. Swelling-activated taurine loss is mediated by a passive [14, 17], Na^+-independent [10–15, 17, 19] transport pathway that does not saturate with taurine concentrations as high as 15 to 60 mM [10, 12–14]. Taurine efflux is blocked by quinidine and quinine [15, 18] and a variety of anion transport inhibitors including DIDS, SITS, NPPB, 1,9-dideoxyforskolin, anthracene-9-carboxylate, furosemide, niflumic acid and MK-196 [10–12, 16–19, 23, 24].

In renal epithelial cells, swelling induces enhanced efflux of sorbitol, myo-inositol, betaine, glycerophosphorylcholine [25–28] and various amino acids [14, 15, 22]. Spring and co-workers have characterized the sorbitol efflux pathway in renal papillary epithelial cells. Cell swelling induces a rapid increase in passive [25, 26] sorbitol efflux from this cell line. Sorbitol loss is unaffected by extracellular Na^+ removal, but is inhibited ~50% by replacement of Cl^- with gluconate [25]. The efflux pathway is located in the apical membrane [26] and does not saturate with sorbitol concentrations up to 315 mM [25]. Sorbitol efflux is blocked by quinidine and the anion transport blockers DIDS, SITS [25] and NPPB [29]. The lipoxygenase/cytochrome P-450 blockers, ketoconazole and SKF-525A, and the acetylenic derivatives of arachidonic acid, ETYA and ETI are also effective inhibitors of sorbitol efflux [27].

Recent studies from this laboratory have characterized the volume-sensitive myo-inositol efflux mechanism in C6 glioma cells, an astrocyte-like cell line derived from rat brain [30]. Myo-inositol efflux is activated rapidly by cell swelling (Fig. 1) and is mediated by a passive transport mechanism that does not saturate with myo-inositol concentrations up to 200 mM. The transport pathway is inhibited by quinidine and quinine and a number of anion transport blockers such as furosemide, SITS, niflumic acid, 1,9-dideoxyforskolin and NPPB, and by the lipoxygenase/cytochrome P-450 inhibitors, ketoconazole, CDC and gossypol. Efflux is also blocked by polyunsaturated fatty acids such as arachidonic acid, linoleic acid and linolenic acid. In addition, the acetylenic derivatives of arachidonic acid, ETYA, ETI, and EDYA block myo-inositol loss. Unsaturated fatty acids are known anion transport blockers [31].

Myo-inositol efflux from C6 cells is also inhibited to some extent by removal of extracellular Na^+ or Cl^- [30]. Replacement of Na^+ with NMDG blocks efflux by ~30%. The degree of inhibition observed with Cl^- removal depends on the substitute that is used.

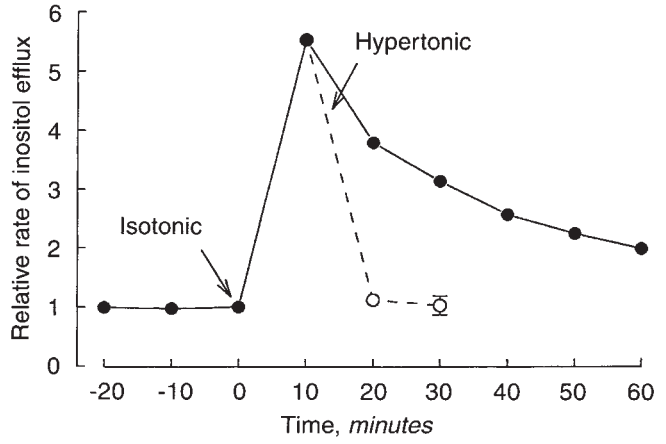


Fig. 1. *^{3}H-myo-inositol efflux is increased transiently when C6 cells acclimated to hypertonic conditions (○–○) are swollen by exposure to isotonic medium (●).* Values are relative to the efflux measured at −20 minutes. ^{3}H-myo-inositol efflux was measured by sampling and replacing the bathing solution at 10 minutes intervals. Arrows indicate when the cells were transferred to either isotonic (solid points) or hypertonic (open points) medium. Solid and open points represent data obtained in experiments carried out on two separate groups of cells. (From [30]; used with permission.)

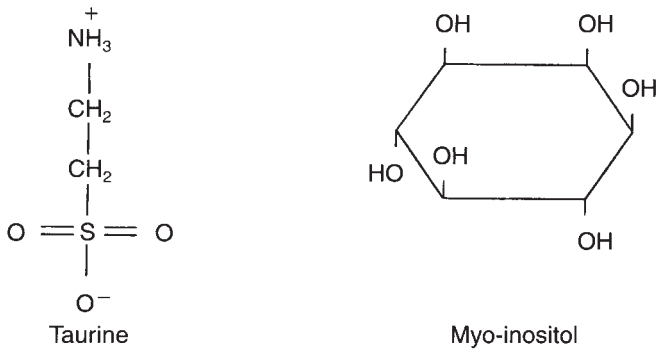


Fig. 2. *Chemical structures of myo-inositol and taurine.*

Subsitution of Cl^- with isethionate or gluconate inhibits myo-inositol loss 80 to 90% while replacement with NO_3^- or SCN^- blocks it by 25 to 30%. The mechanisms by which Na^+ and Cl^- substitution inhibit myo-inositol efflux are unclear [30]. Removal of these ions probably causes significant cell shrinkage that directly inhibits myo-inositol efflux. Removal of Na^+ or Cl^- may also alter intracellular pH, which in turn could inhibit the efflux pathway. Finally, Na^+ and Cl^- may somehow be involved in the regulation of myo-inositol efflux and/or substitutes for these ions may function as partial blockers of the efflux pathway [30].

Volume-sensitive methylamine transport has been characterized in skate erythrocytes. Goldstein and Davis [32] demonstrated recently that Na^+-independent, swelling-activated betaine flux is mediated by a nonsaturable transport pathway that is blocked by DIDS, pyridoxal-5-phosphate, NPPB, MK447A and quinine. In addition, betaine transport is blocked by arachidonic, linoleic and oleic acids.

The above discussion demonstrates that amino acid, polyol and methylamine efflux pathways share a number of characteristics. Efflux of these solutes is mediated by passive, Na^+-independent, very low affinity transport mechanisms, suggesting that a diffusion process is responsible for organic osmolyte loss. In addition, swelling-activated organic osmolyte efflux pathways are all inhibited by anion transport blockers.

The efflux of structurally dissimilar organic osmolytes is mediated by a single transport pathway

The similarities of the amino acid, polyol and methylamine loss pathways observed in diverse organisms suggested to us that a single transport mechanism mediates the efflux of most organic osmolytes from mammalian cells [30]. This hypothesis is supported by several observations. For example, Furlong, Moriyama and Spring [27] demonstrated that myo-inositol, sorbitol and betaine loss from renal papillary cells are all blocked by the same inhibitors of arachidonic acid metabolism. Kirk, Ellory and Young [12] demonstrated recently that the swelling-activated taurine efflux pathway in flounder erythrocytes is permeable to glucose and uridine. In C6 cells, sorbitol and mannitol permeate the myo-inositol efflux pathway [30].

The classical approach for determining whether multiple solutes are transported by a single, carrier-type transport system is to perform competition and/or *trans*-stimulation studies. Competition and *trans*-stimulation have not been observed, however, for organic osmolyte efflux, a result expected if the pathway is a channel [25, 32; Strange and Morrison, unpublished observations]. To test then whether a common transport pathway mediates the efflux of multiple organic osmolytes, we compared the characteristics of taurine efflux in C6 cells to those we had described previously for myo-inositol loss [30]. While this approach was indirect, it was bolstered by the fact that we had identified a large number of experimental maneuvers that substantially inhibit or stimulate the swelling-induced efflux of myo-inositol [30]. We chose to compare the transport charactersitics of taurine and myo-inositol because these solutes are structurally dissimilar (Fig. 2) and because they play important roles in brain osmoregulation [30].

The characteristics of taurine and myo-inositol efflux are effectively identical [33]. The efflux of both solutes exhibits the same nonlinear dependence on cell volume and time course of transient activation in response to swelling. A number of anion transport inhibitors, lipoxygenase/cytochrome P-450 blockers and polyunsaturated fatty acids have nearly identical inhibitory effects on taurine and myo-inositol loss. Forskolin and the phorbol ester PMA have identical stimulatory effects on swelling-activated efflux of these two solutes. In addition, the efflux of both solutes is inhibited to similar degrees by extracellular Na^+ or Cl^- substitution. These findings led us to conclude that a single transport pathway mediates the efflux of structurally unrelated organic osmolytes from cells [33].

A similar conclusion was made recently by Goldstein and Davis [32]. These investigators demonstrated that cell swelling increases taurine, myo-inositol and betaine transport in the skate erythrocyte. The transport characteristics for these three solutes are effectively identical. Betaine, myo-inositol and taurine flux are mediated by a Na^+-independent transport pathway that does not saturate with solute concentrations up to 10 mM. In addition, the transport of all three solutes is inhibited to similar degrees by anion transport blockers, quinine, and unsaturated fatty acids.

Table 1. Molecular dimensions of organic osmolytes

Solute	Length	Width	Thickness
Taurine	7.9	4.0	4.6
Proline	7.2	5	4.9
Myo-inositol	7.2	5.4	5.9
Sorbitol	11.3	5.0	5.0
Betaine	7.1	4.5	5.8
GPC	13.3	5.2	5.7

Dimensions are given in angstroms. Measurements of taurine and proline were taken from Haynes and Goldstein [10]. Dimensions of myo-inositol, sorbitol, betaine and GPC were kindly determined by Mr. Steve Bogusz and Dr. David Busath at Brown University.

The organic osmolyte efflux pathway is a swelling-activated anion channel

The transport characteristics of the organic osmolyte efflux pathway and its inhibition by anion transport blockers and fatty acids suggested indirectly that loss of these solutes is mediated by an anion channel. This hypothesis had first been proposed for swelling-induced taurine and amino acid loss from MDCK cells [14]. Kirk et al [12] proposed a similar hypothesis after they observed that swelling-induced taurine efflux from flounder erythrocytes is blocked by the anion transport inhibitors furosemide, niflumic acid, MK-196, DIDS and NPPB. A compelling argument for the involvement of anion channels in volume-sensitive amino acid efflux was presented recently by Banderali and Roy [22]. These investigators demonstrated the presence of a swelling-activated, outwardly rectifying anion channel with a relatively high permeability to taurine, glutamate and aspartate in MDCK cells. Swelling-activated efflux of these same three amino acids appears to play a quantitatively important role in regulatory volume decrease (RVD) in this cell line [34].

Is it realistic to postulate that an anion channel mediates the efflux of structurally unrelated organic osmolytes from cells? Intuitively, it might seem that these solutes are too big to permeate ion channels. Examination of the molecular dimensions of organic osmolytes indicates otherwise, however. Table 1 shows the length, width and thickness of a number of organic osmolytes used commonly by mammalian cells. If these solutes are viewed as a cylinder, they have *maximal* cylindrical diameters of 4.6 to 5.9 Å. By comparison, Cl^- has a diameter of 3.6 Å [35].

How do the molecular dimensions of anion channels compare with those of organic osmolytes? Halm and Frizzell [35] recently estimated that the minimum pore diameter of the outwardly rectifying apical anion channel in T84 cells is 5.5 to 6.0 Å. Glycine- and GABA-receptor linked Cl^- channels have minimum pore diameters of 5.2 Å and 5.6 Å, respectively [36]. Rasola et al [37] noted that volume-sensitive anion channels in human intestinal and airway epithelial cells have relatively high gluconate and SO_4^{2-} permeabilities indicating a pore diameter >5.4 Å. Thus, it is clearly realistic to envision that organic osmolytes are capable of permeating some types of anion channels.

To test the hypothesis that the organic osmolyte efflux pathway is an anion channel, we carried out whole cell patch clamp studies [33]. C6 cells were grown and patch clamped in hypertonic (390 or 440 mOsm) medium. Cell swelling was induced by reducing bath osmolality to 290 mOsm. Under hypertonic conditions, C6 cells have an extremely low membrane conductance (approximately 0.02 nS/pF). Following cell swelling, however, whole-cell conductance increases rapidly to values up to 1.5 to 2 nS/pF (Fig. 3). This swelling-activated conductance is selective for anions over cations ($P_{cation}/P_{Cl} < 0.04$). The anion permeability sequence of the whole-cell current is $SCN^- > I^- > NO_3^- > Br^- > Cl^- > F^-$ > isethionate > gluconate, which corresponds to Eisenman's sequence I. Swelling-activated anion currents show outward rectification, are inactivated by membrane potentials above +60 mV (Fig. 3) [33, 38] and are blocked by extracellular nucleotides such as ATP [38].

If swelling-activated anion channels mediate organic osmolyte efflux, then whole cell conductance should be blocked by inhibitors of the efflux pathway. Exposure of cells to 100 μM ketoconazole, 50 μM arachidonic acid, 100 μM NPPB, 100 μM cinnamyl-3,4-dihydroxy-a-cyanocinnamate, or 100 μM 1,9-dideoxyforskolin causes a rapid (< 60 seconds), 80 to 100% inhibition of swelling-activated whole cell Cl^- currents. Swelling-activated myo-inositol and taurine efflux are inhibited to a similar degree by these drugs (Fig. 4) [33]. Two agents that do not alter organic osmolyte efflux, verapamil and oleic acid, also do not inhibit whole cell Cl^- currents [33, 39].

If volume-sensitive Cl^- channels permeable to myo-inositol and taurine are activated in intact C6 cells, then cell swelling should cause an increase in Cl^- loss that can be blocked by inhibitors of organic osmolyte efflux. Cell swelling induces a two- to threefold increase in the rate of $^{36}Cl^-$ efflux that is blocked completely by 100 μM ketoconazole [33].

Taurine is a zwitterion and at physiological pH (that is, 7.4) ~4% of it is negatively charged. Elevation of pH increases the proportion of anionic taurine in solution. If taurine permeates swelling-activated anion channels, then it should be possible to measure taurine currents when bath Cl^- is replaced by this solute. Replacement of bath Cl^- with a taurine solution at pH 8.2 (57 mM negatively charged taurine) shifts the reversal potential (E_{rev}) of the whole-cell anion current by ~+50 mV. The relative taurine permeability of the conductance compared to Cl^- is ~0.2. These measurements demonstrate directly that swelling-activated anion channels are capable of mediating taurine efflux [22, 33].

In an effort to obtain further evidence for the movement of polyols through swelling-activated anion channels, we exposed C6 cells to high extracellular levels of myo-inositol or sorbitol. Cells were patch clamped with bath and pipet solutions containing 25 mM CsCl. The osmolality of the solutions was adjusted by addition of the trisaccharide, raffinose. After activation of the whole-cell anion conductance by cell swelling, the membrane potential was clamped at 0 mV and the raffinose in the bath was replaced by 210 mM myo-inositol or 210 mM sorbitol. This experimental maneuver induces transient, 5 to 20 pA inward currents. When the cells are returned to a raffinose medium, a transient outward current is observed. These transient currents are only seen when switching between solutions containing the impermeant solute raffinose, and the permeant solutes myo-inositol and sorbitol. Exposure of the cells to 100 μM ketoconazole or 100 μM NPPB causes a nearly complete and at least partially reversible blockage of polyol-induced currents. The effects of polyols on current flow through the channel suggest that these solutes compete with Cl^- for common channel binding sites [33].

The experimental observations described above provide strong evidence in support of the hypothesis that a swelling-activated anion channel mediates the efflux of multiple organic osmolytes from cells. Whole cell currents generated by this channel have

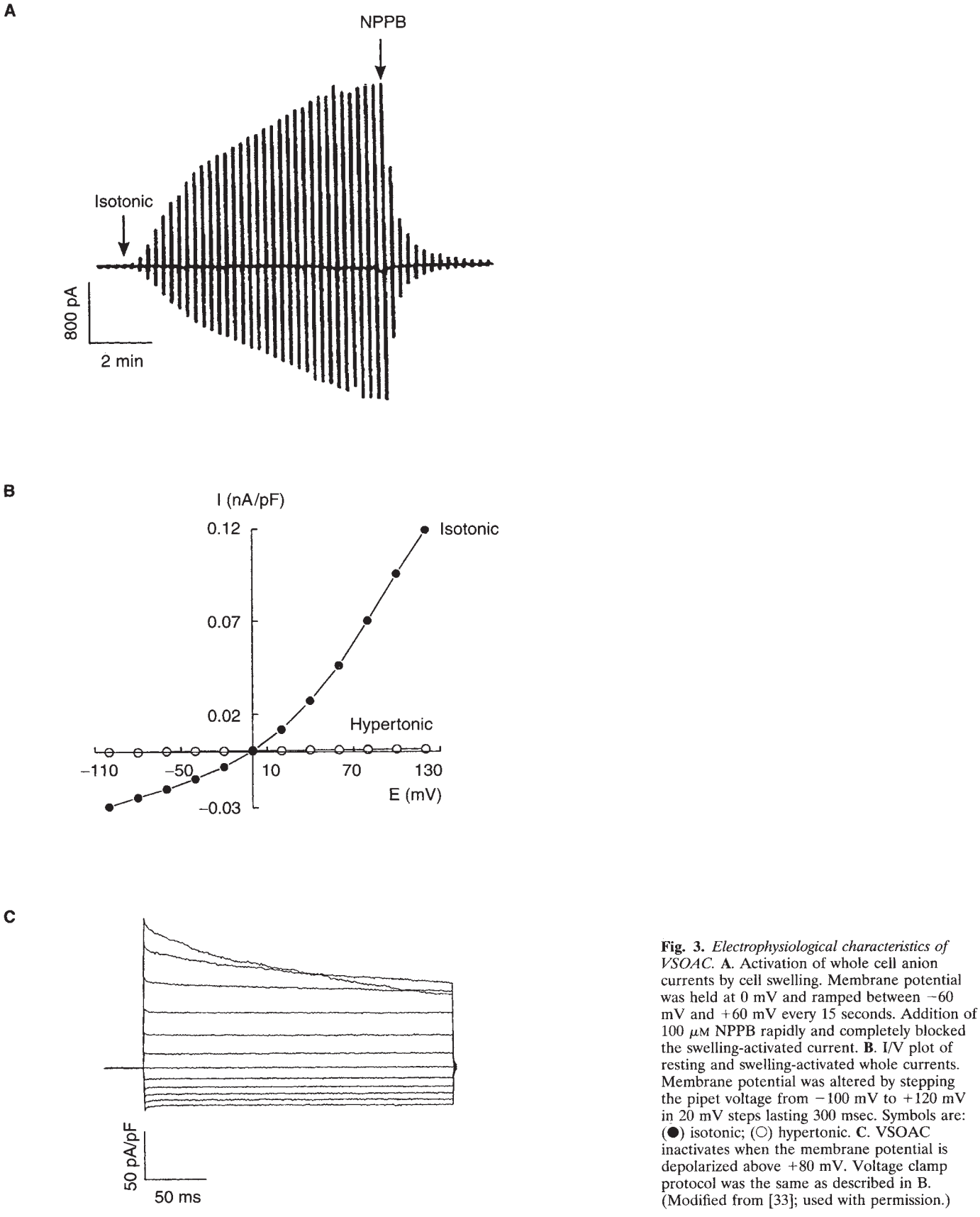


Fig. 3. *Electrophysiological characteristics of VSOAC.* **A.** Activation of whole cell anion currents by cell swelling. Membrane potential was held at 0 mV and ramped between −60 mV and +60 mV every 15 seconds. Addition of 100 μM NPPB rapidly and completely blocked the swelling-activated current. **B.** I/V plot of resting and swelling-activated whole currents. Membrane potential was altered by stepping the pipet voltage from −100 mV to +120 mV in 20 mV steps lasting 300 msec. Symbols are: (●) isotonic; (○) hypertonic. **C.** VSOAC inactivates when the membrane potential is depolarized above +80 mV. Voltage clamp protocol was the same as described in B. (Modified from [33]; used with permission.)

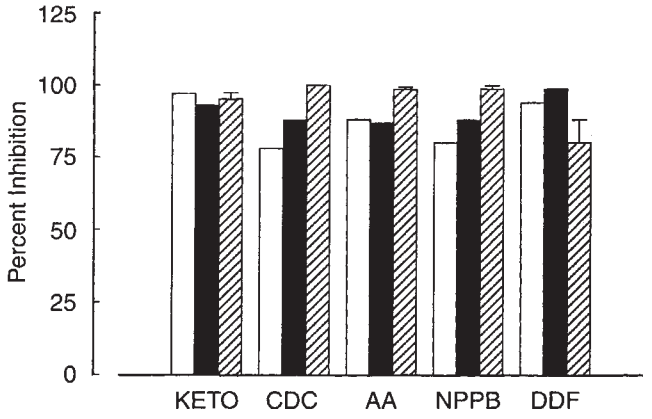


Fig. 4. *VSOAC and swelling-activated myo-inositol and taurine efflux are blocked to similar extents by 100 μM ketoconazole (KETO), 100 μM CDC, 50 μM arachidonic acid (AA), 100 μM NPPB or 100 μM 1,9-dideoxyforskolin (DDF).* Symbols are: (□) inositol; (■) taurine; (▨) Cl current. (From [33]; used with permission.)

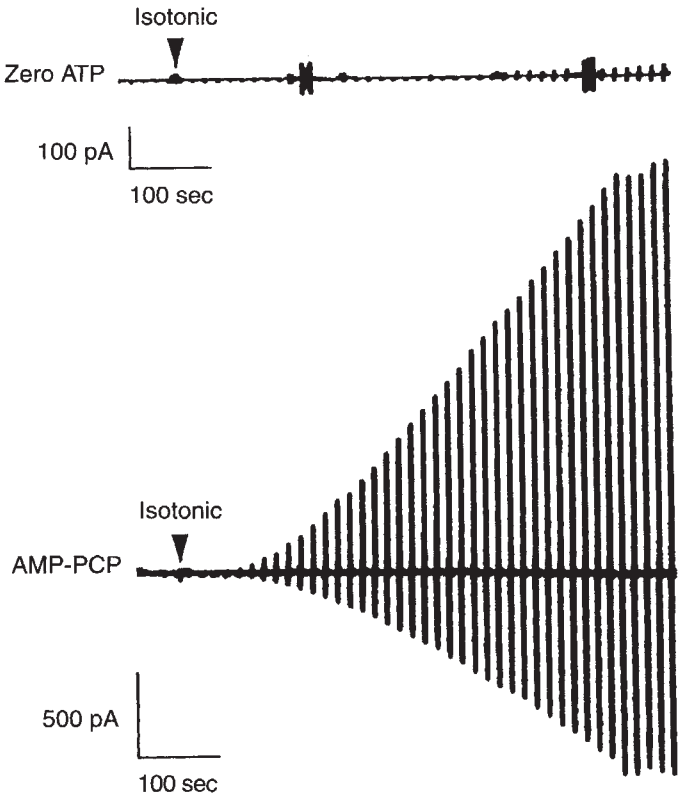


Fig. 5. *Swelling-induced activation of VSOAC requires the presence of intracellular ATP or non-hydrolyzable ATP analogs.* Cells were dialyzed for 5 to 8 minutes before swelling with either an ATP-free patch pipet solution or one containing 1 mM AMP-PCP. The voltage clamp protocol was the same as that described in Figure 3A.

characteristics similar to those of other volume-sensitive anion conductances described in a variety of cell types including airway [37, 40–42], intestinal [37, 42–44] and ciliary [45] epithelial cells, keratinocytes [46], lymphocytes [47], neutrophils [48] and *Xenopus* oocytes [49]. The whole cell anion conductance activated by volume increases in these cells is outwardly rectifying [37, 40–42, 44–49], blocked by anion transport inhibitors such as SITS and NPPB, as well as arachidonic acid [37, 41, 43, 45–49], and has relatively high permeability to various inorganic and organic anions [37, 43, 47–48]. Many of the conductances described are also voltage-sensitive, exhibiting pronounced inactivation at positive membrane potentials from +60 to +120 mV [37, 38, 40–42, 44, 46, 49].

The similarity of the swelling-activated whole cell anion conductance in C6 cells to those described in numerous other cell types suggests that its proposed role in organic osmolyte efflux is not unique to this cell line. Instead, we postulate that outwardly rectifying, volume-sensitive anion channels responsible for swelling-activated whole cell currents play a ubiquitous role in organic osmolyte regulation. Since mammalian cells appear to possess several types of volume-sensitive anion channels (discussed below), we have termed the channel responsible for the outwardly rectifying whole cell current, VSOAC for volume-sensitive organic osmolyte-anion channel.

Is VSOAC or a related channel involved in organic osmolyte efflux in non-mammalian cells? In *Xenopus* oocytes, a swelling-activated whole cell current with the characteristics of VSOAC has been described [49]. Recent studies from this laboratory have characterized a swelling-activated anion conductance in hepatocytes of the marine skate *Raja erinacea* [50]. The channel responsible for the conductance in skate hepatocytes is permeable to taurine and has functional characteristics identical to those of the swelling-activated taurine efflux pathway described in these cells [11, 50, 51].

Studies in skate erythrocytes by Goldstein and co-workers have suggested the involvement of the band 3 anion exchanger in organic osmolyte loss [10, 21, 32]. As in all cells in which it has been studied, the swelling-induced flux of these solutes in skate erythrocytes appears to be mediated by a channel. Indeed, Haynes and Goldstein [10] measured Na^+-independent swelling-activated influx of various amino acids into skate red cells and estimated that the pore size of the putative channel is between 5.7 and 6.3 Å in diameter. Goldstein and Davis [32] have suggested that this channel may be a component of the band 3 molecule or may somehow be regulated by the anion exchanger.

VSOAC is regulated by cell metabolic state

Dialysis of C6 cells for five to eight minutes with an ATP-free patch pipet solution prevents or dramatically inhibits swelling-induced activation of VSOAC. Replacement of ATP with poorly (ATP-γ-S) or non-hydrolyzable (AMP-PCP or AMP-PNP) ATP analog restores normal channel activation (Fig. 5) [39]. Removal of GTP has no effect on swelling-induced whole-cell currents. The requirement for ATP is specific for the triphosphate moiety; 2 mM ADP or 2 mM AMP do not support channel activation [39]. Activation of the volume-sensitive anion conductance in skate hepatocytes has a similar requirement for ATP or non-hydrolyzable ATP analogs [50].

VSOAC activates normally for three to five minutes in the presence of an ATP-free patch pipet solution as long as cell swelling is induced immediately after membrane rupture. As cellular ATP is dialyzed away, however, channel activity declines slowly [39]. Apparent ATP-dependent rundown of swelling-activated anion currents has also been observed in T lymphocytes

[47], neuroblastoma cells [52] and endothelial cells [53]. In C6 cells [39] and endothelial cells [53], rundown is prevented by inclusion of a non-hydrolyzable ATP analog in the patch pipet [39].

Consistent with the ATP dependence of VSOAC activation, we observed that passive, swelling-induced efflux of myo-inositol and taurine from C6 cells is inhibited substantially (> 90%) by several metabolic inhibitors that lower intracellular ATP levels [39]. Titration of cellular ATP with azide revealed that myo-inositol and taurine efflux have an identical dependence on ATP concentration. The apparent K_d for ATP is ~1.7 mM [39]. Swelling-activated taurine efflux from skate hepatocytes shows a similar dependence on cellular ATP levels [51]. The ATP concentration in most cells is maintained between 3 to 8 mM [54]. Under pathologic conditions, ATP levels can drop to micromolar concentrations in some cells and tissues (such as brain) [55, 56]. The high K_d for ATP indicates that cellular metabolic state plays an important role in modulating passive organic osmolyte efflux.

The dependence of organic osmolyte efflux on cellular ATP levels has important physiological, clinical and ecological implications [39]. Organic osmolytes are metabolically expensive to accumulate [2]. For example, when adapted to 390 mOsm culture medium, C6 cells transport myo-inositol into the cytoplasm against a 6,000- to 9,000-fold concentration gradient [57]. The intertidal bivalve *Mytilus californianus* maintains transmembrane taurine gradients of > 1 million fold [58]. Since organic osmolytes represent a significant fraction of the total intracellular osmolality [1, 3], and since they may play important 'cytoprotective' roles [4], it is advantageous to reduce passive loss of these solutes when cellular energy production is reduced.

VSOAC is highly permeable to important metabolic itermediates such pyruvate, the short-chain fatty acids acetate and butyrate, the ketone body β-hydroxybutyrate and several amino acids (relative permeability ~0.2 to 0.4) [39]. These metabolites are major inputs into the TCA cycle and excessive loss of them could conceivably disrupt cellular energy production. Modulation of channel activity by cellular ATP levels provides essential feedback regulation that prevents depletion of energy producing carbon sources when cellular energy production is reduced.

In crab axons [8], skate hepatocytes [11] and red cells of the blood clam [9], swelling-induced taurine and amino acid efflux and volume regulation are blocked by metabolic inhibitors. Modulation of passive organic osmolyte loss by cellular ATP levels may therefore be a widespread phenomenon and represent an adaptation to osmotically unstable environments. Certain organisms such as intertidal invertebrates experience extreme and daily cyclical fluctuations in salinity requiring cells to repeatedly lose and reaccumulate organic osmolytes. It is not surprising to find, therefore, that the metabolic costs of cell volume regulation in intertidal invertebrates are high [59]. These metabolic costs are exacerbated by periods of O_2 deprivation that are sometimes associated with fluctuations in salinity. Interestingly, conditions analogous to those experienced by intertidal invertebrates, repetitive shifts in extracellular osmolality [3] and transient periods of anoxia [60], occur in the renal medulla. It may be advantageous to link passive organic osmolyte loss to cellular ATP levels in organisms and cells experiencing cyclical osmotic and metabolic stress. Such a linkage would minimize the energy costs required to reaccumulate organic osmolytes when cells are re-exposed to hypertonic conditions. It seems likely that the ATP dependence of VSOAC is a primitive adaptation that allows cells to cope with competing demands of volume control, maintenance of proper intracellular organic osmolyte levels and preservation of energy metabolism.

VSOAC is regulated by cellular Cl^- levels

Recent studies in skate hepatocytes have revealed that activation of VSOAC is inversely related to cellular Cl^- levels [50]. Chloride and possibly ionic strength appear to modulate the volume set-point of the channel. When intracellular Cl^- levels are high, larger increases in cell volume are required to induce a given amount of VSOAC activity than when cell Cl^- levels are low. These results are consistent with studies of Motais and co-workers demonstrating that taurine efflux from trout red blood cells is inhibited by increases in intracellular ionic strength [61]. The Cl^- sensitivity of VSOAC allows cells to conserve organic osmolytes if Cl^- levels are sufficient to mediate recovery from a given volume increase. Furthermore, it prevents additional increases in intracellular ionic strength if cells are swollen by electrolyte accumulation. In the presence of high intracellular Cl^- levels, cells may activate more Cl^--selective transport pathways to mediate volume regulation [62].

What is the main function of VSOAC?

It is difficult to envision why a swelling-activated channel responsible primarily for moving Cl^- should be regulated by cellular ATP levels and inhibited by high intracellular Cl^- levels. ATP-dependent regulation and sensitivity to cell Cl^- are clearly advantageous, however, if the channel's principal role is to mediate the passive efflux of metabolically expensive, 'cytoprotective' organic osmolytes. We suggest that the major function of VSOAC is organic osmolyte transport.

How is VSOAC activated in response to cell swelling?

At present, there is little understanding of how cells "sense" volume and transduce "size" signals into various regulatory responses. Similarly, we do not know how VSOAC is activated by cell swelling. Studies in C6 cells, however, have ruled out a number of possible signal transduction pathways that could be involved [30, 63]. Intracellular Ca^{2+} levels (Ca^{2+}_i), cGMP, G proteins [30] and arachidonic acid metabolites [63] do not regulate VSOAC. Exposure of C6 cells to 20 nM PMA, 0.5 mM 8-bromo-cAMP or 50 μM forskolin has no effect on the basal rate of myo-inositol loss, but stimulates swelling-induced efflux by two- to threefold. The stimulatory effects of cAMP and PMA are additive [30]. Treatment of cells with the protein kinase inhibitors, H-7 or staurosporine, or down-regulation of PKC activity, however, has no inhibitory effect on myo-inositol efflux and cellular cAMP levels are not altered by cell swelling. These results indicate that stimulation of PKC or PKA modulates the activity of VSOAC, but is not required for swelling-induced activation [30].

Cells possess several different types of volume-sensitive anion channels

It appears likely that cells possess several different types of volume-sensitive anion channels. For example, Jentsch and coworkers [64, 65] have cloned an anion channel from rat brain referred to as ClC-2. When expressed in *Xenopus* oocytes, the

channel is activated by cell swelling and membrane hyperpolarization [64, 65] and is selective for Cl^- over other anions ($Cl^- \geq Br^- > I^-$) [63]. The instantaneous current-to-voltage relationship shows slight inward rectification [65]. Northern analysis of diverse cell types suggests that ClC-2 is distributed ubiquitously [64].

A "maxi" volume-sensitive anion channel has been described in rat cortical astrocytes [66], cultured renal cortical collecting duct (CCD) cells [67] and in neuroblastoma cells [68]. The unitary conductance of this channel is 200 to 400 pS and the current-voltage relationship is linear. Channel opening is voltage dependent. In astrocytes, for example, the open probability of the channel is low when the membrane potential is outside the range of −40 to +40 mV. Between these voltages, however, channel opening increases dramatically and is highest at 0 mV [66]. The channel displays a similar voltage dependence in CCD [67] and neuroblastoma cells [68].

Single channel studies have revealed the presence of small conductance swelling-activated anion channels in Ehrlich ascites cells [69] and choroid plexus [70]. These channels have a unitary conductance of 2 to 7 pS. In Ehrlich cells, the current-voltage relationship for the channel is linear [69]. A 28 pS swelling-acitvated Cl^- channel with a linear current-voltage relationship has been observed in rat colonic epithelium [71].

Recent studies of Sepúlveda and co-workers have suggested that P-glycoprotein, the product of the multidrug resistance (MDR1) gene, functions as a both an ATP-dependent pump and a volume-sensitive Cl^- channel [72–74]. Swelling-activated whole-cell currents postulated to be due to P-glycoprotein are effectively identical to those arising from VSOAC [33, 39, 72–74]. The possibility that a single membrane protein functions as both a pump and channel is intriguing. Unfortunately, a number of studies from several different laboratories [75–79], including our own [39] have failed to correlate cell volume regulatory properties and whole cell, volume-sensitive Cl^- currents with P-glycoprotein expression. In certain types of cells, changes in P-glycoprotein expression may regulate or modify the activation of volume-sensitive anion channels such as VSOAC [76]. It is also possible that experimental maneuvers designed to vary P-glycoprotein expression may indirectly alter channel expression and activation.

'Mini' and 'intermediate' conductance volume-sensitive anion channels have also been described. Both channel types have been proposed to be responsible for whole cell, swelling-activated, outwardly rectifying anion currents. Stationary noise analysis has suggested that the channel has a unitary conductance of 1 to 2 pS in T lymphocytes [47] and neutrophils [48]. In contrast, single channel measurements in epithelial cells have demonstrated the existence of a swelling-induced, outwardly rectifying, depolarization-inactivated channel with a unitary conductance of 40 to 90 pS at strongly depolarizing voltages [42, 44, 80]. It is possible that different channels are responsible for the swelling-activated anion currents observed in different cell types. Recent studies from our laboratory (discussed below), however, have demonstrated that stationary noise analysis significantly underestimates the unitary conductance of outwardly rectifying, swelling activated anion channels in C6 glioma cells [81].

Unitary properties of VSOAC

To characterize the unitary properties of VSOAC, we performed noise analysis and single channel measurements [81]. Current noise was analyzed initially by assuming that graded changes in macroscopic current were due to graded changes in channel open probability. Stationary noise analysis of swelling-activated anion currents indicate that VSOAC has a unitary conductance of ~0.5 pS at −50 mV and that there are ~60,000 to 70,000 channels/cell. In sharp contrast, nonstationary noise analysis of anion currents during depolarization-induced inactivation predicts that VSOAC has a conductance ~45 pS at +120 mV and that there are ~1,000 to 3,000 channels/cell.

Single channel measurements confirm the nonstationary noise analysis results [81]. Single channel closures are observed during steps to depolarizing voltages in outside-out membrane patches pulled from swollen cells. In addition, single channel, depolarization-induced closures are observed in whole cell currents recorded during the very early stages of cell swelling. The unitary conductance of VSOAC determined from these closures is 40 to 50 pS at +120 mV.

Once activated by cell swelling, the open probability of VSOAC is near unity [42, 80, 81]. In addition, when the channel spontaneously closes, it rapidly re-opens. Spectral analysis of whole cell currents indicates that the mean closed time for VSOAC is ~0.3 ms.

Stationary noise analysis underestimates the unitary conductance of VSOAC by at least 15-fold because swelling-induced current activation is not mediated by graded increases in channel open probability. Instead, activation of VSOAC appears to involve an abrupt switching of single channels from an *OFF* state, where channel open probability is zero, to an *ON* state, where open probability is near unity [81].

A molecular and cellular model of VSOAC activation

In light of the studies reviewed above, it is interesting and important to examine the recent work of Paulmichl, Clapham and coworkers [82, 83]. Paulmichl et al [82] have described the cloning of a cDNA termed I_{Cln}. When overexpressed in *Xenopus* oocytes, I_{Cln} gives rise to an anion conductance that is constitutively active, outwardly rectifying, blocked by conventional anion channel inhibitors, inactivated by strong depolarization, and blocked by external ATP and other nucleotides. The I_{Cln}-induced conductance is thus indistinguishable from whole cell currents induced by activation of VSOAC [33, 38, 81]. Mutations of a putative nucleotide binding site on the I_{Cln} protein prevents inhibition by extracellular nucleotides, alters channel voltage-dependence and confers sensitivity to external Ca^{2+} [82]. Based on these findings, it was concluded that I_{Cln} codes for an anion channel. Structural analysis suggests that the 235 amino acid I_{Cln} protein forms four β strands but no transmembrane helices. Paulmichl et al [82] proposed that the putative channel consists of a dimer of the protein with the pore being formed by an eight-stranded antiparallel β barrel.

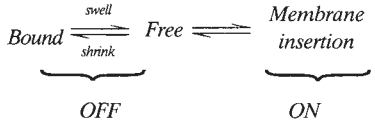
Krapivinsky et al [83] demonstrated recently that I_{Cln} codes for an abundant, soluble, highly acidic protein located primarily in the cytoplasm. This protein forms tight oligomeric complexes with other cytoplasmic proteins including actin. The cytoplasmic location and biochemical characteristics of the I_{Cln} protein are unexpected for a channel protein. In addition, Ackerman, Wickman and Clapham [49] demonstrated that *Xenopus* oocytes possess a swelling-activated anion current with characteristics identical to those of VSOAC and to those induced by overexpression of I_{Cln}. Based on these findings, Krapivinsky et al [83] proposed that the I_{Cln} protein is not an anion channel, but a channel regulator.

Overexpression of I_{Cln} in oocytes was postulated to activate the endogenous volume-sensitive anion conductance [83].

The disparate conclusions made by these two groups of investigators are difficult to reconcile given current models of channel regulation. This indicates that alternate models should be examined. As a starting point, it is important to consider the proposed β barrel structure of the I_{Cln} protein [82]. Beta barrels are a defining structural characteristic of porins, a primitive class of channels that play a critical role in organic solute transport across bacterial outer membranes [84–86]. Porins are soluble, highly acidic proteins [84–86]. Shortly after synthesis, the proteins are transported across the cytoplasmic membrane into the periplasmic space where they then spontaneously insert into the outer membrane [86]. Once inserted into a lipid bilayer, porins have a very high open probability and exhibit only very brief spontaneous channel closures [84, 85, 87, 88].

We believe that the simplest hypothesis given the available data is that VSOAC is coded for by the I_{Cln} gene. The proposed β barrel structure of the I_{Cln} protein implies that the functional characteristics of the channel might be 'porin-like' in nature. This idea is consistent with the observation that VSOAC, like porins [84, 85], is permeable to a wide variety of structurally dissimilar organic solutes [22, 33, 39]. The finding that the open probability of VSOAC is near unity [42, 80] and spontaneous channel closures are very brief [84, 85, 87, 88] also suggest that the channel is 'porin-like.'

Assuming then that VSOAC and the I_{Cln} protein are synonymous, a cellular model that describes channel activation within the context of the results presented here and by Paulmichl, Clapham and coworkers [82, 83] can be proposed. As discussed above, activation of VSOAC appears to involve an abrupt switching of the channel from an *OFF* state where open probability is 0 to an *ON* state where open probability is near unity. In our model, the *OFF* state would represent the I_{Cln}/VSOAC channel when it is located in the cytoplasm. This putative cytoplasmic localization would be maintained by binding or 'anchoring' of the channel protein to other cellular proteins such as those described by Krapivinsky et al [83]. In response to swelling, I_{Cln}/VSOAC would be released from its cytoplasmic anchoring site where it would then be free to insert spontaneously into the plasma membrane. Membrane insertion represents the abrupt switching of the channel into the *ON* state. This 'anchor-insertion' model is illustrated schematically below.



Clearly, extensive studies involving reconstitution, site-directed mutagenesis and immunocytochemsitry approaches are required to test the 'anchor-insertion' model and to determine whether VSOAC is coded for by the I_{Cln} gene.

Conclusions and future perspectives

Organic osmolytes are used universally by cells for maintenance of osmotic homeostasis. In many different organisms and cell types, cell swelling induces a rapid efflux of these solutes to the extracellular medium. The loss of organic osmolytes from cells is mediated by Na^+-independent, very low affinity, passive transport pathways that are blocked by a variety of anion channel inhibitors. In C6 glioma cells we have demonstrated that the efflux of multiple, structurally unrelated organic osmolytes occurs via a ubiquitously distributed, volume-sensitive, ATP-dependent anion channel we have termed VSOAC. Accumulating evidence in diverse organisms and cell types suggests that volume-sensitive anion channels play a widespread role in organic osmolyte homeostasis.

The electrophysiological characteristics of VSOAC differentiate it clearly from other volume-sensitive anion channels that have been described. What are the relationships between these various channels and what roles do they play in volume regulation and other cellular functions? Is their activity modulated by factors other than cell volume? Do these channels have different volume set-points and are those set-points under dynamic regulation? These and other important questions need to be addressed. Perhaps ClC-2 [64, 65] activates in response to small volume increases such as those that can occur during changes in transmembrane solute flux. Since ClC-2 is selective for Cl^- over other anions [64], it would control cell volume primarily by mediating Cl^- loss. VSOAC in contrast may be activated by larger volume perturbations [30, 33], providing a means for the cell to lose large quantities of osmotically active solute rapidly. The presence of volume-sensitive anion channels with different set-points and selectivities to various solutes could provide cells with a mechanism to conserve metabolically expensive, cytoprotective organic osmolytes when faced with only minor volume increases.

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References

1. YANCEY PH: Compatible and counteracting solutes, in *Cellular and Molecular Physiology of Cell Volume Regulation*, edited by K STRANGE, Boca Raton, CRC, 1994, p 81
2. CHAMBERLIN ME, STRANGE K: Anisosmotic volume regulation: A comparative view. *Am J Physiol (Cell Physiol 26)* 257:C159–C173, 1989
3. GARCIA-PEREZ A, BURG MB: Renal medullary organic osmolytes. *Physiol Rev* 71:1081–1115, 1991
4. WRIGHT CE, TALLAN HH, LIN YY, GAULL GE: Taurine: Biological update. *Ann Rev Biochem* 55:427–453, 1986
5. BERRIER C, COULOMBE A, SZABO I, ZORATTI M, GHAZI A: Gadolinium ion inhibits loss of metabolites induced by osmotic shock and large stretch-activated channels in bacteria. *Eur J Biochem* 206:559–565, 1992
6. KIRST GO: Coordination of ionic relations and mannitol concentrations in the euryhaline unicellular alga. *Platymonas subcordiformis* (Hazen) after osmotic shocks *Planta Berl* 135:69–75, 1977
7. HANDA S, HANDA AK, HASEGAWA PM, BRESSAN RA: Proline accumulation and the adaptation of cultured plant cells to water stress. *Plant Physiol* 80:938–945, 1986

8. GERARD JF: Volume regulation and alanine transport response of isolated axons of *Callinectes sapidus* Rathbun to hypo-osmotic conditions. *Comp Biochem Physiol* 51A:225–229, 1975
9. AMENDE LM, PIERCE SK: Free amino acid mediated volume regulation of isolated *Noetia ponderosa* red blood cells: Control by Ca^{2+} and ATP. *J Comp Physiol* 138:291–298, 1980
10. HAYNES JK, GOLDSTEIN L: Volume-sensitive amino acid transport in erythrocytes of the little skate, *Raja erinacea*. *Am J Physiol (Reg Integr Comp Physiol 34)* 265:R173–R179, 1993
11. BALLATORI N, BOYER JL: Taurine transport in skate hepatocytes II. Volume activation, energy and sulfhydryl dependence. *Am J Physiol (Gastrointest Liver Physiol 25)* 262:G451–G460, 1992
12. KIRK K, ELLORY JC, YOUNG JD: Transport of organic substrates via a volume-activated channel. *J Biol Chem* 267:23475–23478, 1992
13. FINCHAM DA, WOLOWYK MW, YOUNG JD: Volume-sensitive taurine transport in fish erythrocytes. *J Membr Biol* 96:45–56, 1987
14. ROY G, MALO C: Activation of amino acid diffusion by a volume increase in cultured kidney (MDCK) cells. *J Membr Biol* 130:83–90, 1992
15. SANCHEZ OLEA R, Pasantes-Morales H, Lazaro A, Cereijido M: Osmolarity-sensitive release of free amino acids from cultured kidney cells (MDCK). *J Membr Biol* 121:1–9, 1991
16. KIMELBERG K, GODERIE SK, HIGMAN S, PANG S, WANIEWSKI RA: Swelling-induced release of glutamate, aspartate, and taurine from astrocyte cultures. *J Neurosci* 10:1583–1591, 1990
17. SANCHEZ OLEA R, Moran J, Schousboe A, Pasantes-Morales H: Hyposmolarity-activated fluxes of taurine in astrocytes are mediated by diffusion. *Neurosci Lett* 130:233–236, 1991
18. KIRK K, KIRK J: Volume regulatory taurine release from a human lung cancer cell line: Evidence for amino acid transport via a volume-activated chloride channel. *FEBS Lett* 336:153–158, 1993
19. JESUS GARCIA J, SANCHEZ OLEA R, PASANTES-MORALES H: Taurine release associated to volume regulation in rabbit lymphocytes. *J Cell Biochem* 45:207–212, 1991
20. LAMBERT IH, HOFFMANN EK: Regulation of taurine transport in Ehrlich ascites tumor cells. *J Membr Biol* 131:67–79, 1993
21. MUSCH MW, LEFFINGWELL TR, GOLDSTEIN L: Band 3 modulation and hypotonic-stimulated taurine efflux in skate erythrocytes. *Am J Physiol (Reg Integr Comp Physiol 35)* 266:R65–R74, 1994
22. BANDERALI U, ROY G: Anion channels for amino acids in MDCK cells. *Am J Physiol (Cell Physiol 32)* 263:C1200–C1207, 1992
23. PASANTES-MORALES H, MORAN J, SCHOUSBOE A: Volume-sensitive release of taurine from cultured astrocytes: properties and mechanism. *Glia* 3:427–432, 1990
24. SANCHEZ OLEA R, PENA C, MORAN J, PASANTES-MORALES H: Inhibition of volume regulation and efflux osmoregulatory amino acids by blockers of Cl^- transport in cultured astrocytes. *Neurosci Lett* 156: 141–144, 1993
25. SIEBENS AW, SPRING KR: A novel sorbitol transport mechanism in cultured renal papillary epithelial cells. *Am J Physiol (Renal Fluid Electrol Physiol 26)* 257:F937–F946, 1989
26. GARTY H, FURLONG TJ, ELLIS DE, SPRING KR: Sorbitol permease: an apical membrane transporter in cultured renal papillary epithelial cells. *Am J Physiol (Renal Fluid Electrol Physiol 29)* 260:F650–F656, 1991
27. FURLONG TJ, MORIYAMA T, SPRING KR: Activation of osmolyte efflux from cultured renal papillary epithelial cells. *J Membr Biol* 123:269–277, 1991
28. BAGNASCO SM, MONTROSE MH, HANDLER JS: Role of calcium in organic osmolyte efflux when MCDK cells are shifted from hypertonic to isotonic medium. *Am J Physiol (Cell Physiol 33)* 264:C1165–C1170, 1993
29. NAPATHORN S, SPRING KR: Futher characterization of the sorbitol permease in PAP-HT25 cells. *Am J Physiol (Cell Physiol 36)* 267: C514–C519, 1994
30. STRANGE K, MORRISON R, SHRODE L, PUTNAM R: Mechanism and regulation of swelling-activated inositol efflux in brain glial cells. *Am J Physiol (Cell Physiol 34)* 265:C244–C256, 1993
31. ORDWAY RW, SINGER JJ, WALSH JV: Direct regulation of ion channels by fatty acids. *Trends Neurol Sci* 3:96–100, 1991
32. GOLDSTEIN L, DAVIS EM: Taurine, betaine and inositol share a volume-sensitive transporter in skate erythrocyte cell membrane. *Am J Physiol (Reg Integr Comp Physiol)* (in press)
33. JACKSON PS, STRANGE K: Volume-sensitive anion channels mediate swelling-activated inositol and taurine efflux. *Am J Physiol (Cell Physiol 34)* 265:C1489–C1500, 1993
34. ROY C, MALO C: Activation of amino acid diffusion by a volume increase in cultured kidney (MDCK) cells. *J Membr Biol* 130:83–90, 1992
35. HALM DR, FRIZZELL RA: Anion permeation in an apical membrane chloride channel of a secretory epithelial cell. *J Gen Physiol* 99:339–366, 1992
36. BORMANN J, HAMILL OP, SAKMANN B: Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurons. *J Physiol* 385:243–286, 1987
37. RASOLA A, GALIETTA LJV, GRUENERT DC, ROMEO G: Ionic selectivity of volume-sensitive curents in human epithelial cells. *Biochim Biophys Acta* 1139:319–323, 1992
38. JACKSON PS, STRANGE K: Characterization of the voltage-dependent properties of a volume-sensitive anion conductance. *J Gen Physiol* 105:661–677, 1995
39. JACKSON PS, MORRISON R, STRANGE K: The volume-sensitive organic osmolyte channel VSOAC is regulated by non-hydrolytic ATP binding. *Am J Physiol (Cell Physiol)* 267:C1203–C1209, 1994
40. CHAN H-C, GOLDSTEIN J, NELSON DJ: Alternate pathways for chloride conductance activation in normal and cystic fibrosis airway epithelial cells. *Am J Physiol (Cell Physiol 31)* 262:C1273–C1283, 1992
41. MCCANN JD, LI M, WELSH MJ: Identification and regulation of whole-cell chloride currents in airway epithelium. *J Gen Physiol* 94:1015–1036, 1989
42. SOLC CK, WINE JJ: Swelling-induced and depolarization-induced Cl^- channels in normal and cystic fibrosis epithelial cells. *Am J Physiol (Cell Physiol 30)* 261:C658–C674, 1991
43. KUBO M, OKADA Y: Volume-regulatory Cl^- channel currents in cultured human epithelial cells. *J Physiol* 456:351–371, 1992
44. WORRELL RT, BUTT AG, CLIFF WH, FRIZZELL RA: A volume-sensitive chloride conductance in human colonic cell line T84. *Am J Physiol (Cell Physiol 25)* 256:C1111–C1119, 1989
45. YANTORNO RE, CARRE DA, COCA-PRADOS M, KRUPIN T, CIVAN MM: Whole cell patch clamping of ciliary epithelial cells during anisosmotic swelling. *Am J Physiol (Cell Physiol 31)* 262:C501–C509, 1992
46. RUGOLO M, MASTROCOLA T, DE LUCA M, ROMEO G, GALIETTA LJV: A volume-sensitive chloride conductance revealed in cultured human keratinocytes by $^{36}Cl^-$ efflux and whole-cell patch clamp recording. *Biochim Biophys Acta* 1112:39–44, 1992
47. LEWIS RS, ROSS PE, CALAHAN MD: Chloride channels activated by osmotic stress in T lymphocytes. *J Gen Physiol* 101:801–826, 1993
48. STODDARD JS, STEINBACH JH, SIMCHOWITZ L: Whole cell Cl^- currents in human neutrophils induced by cell swelling. *Am J Physiol (Cell Physiol 34)* 265:C156–C165, 1993
49. ACKERMAN MJ, WICKMAN KD, CLAPHAM DE: Hypotonicity activates a native chloride current in *Xenopus* oocytes. *J Gen Physiol* 103:153–179, 1994
50. JACKSON PS, CHURCHWELL K, BALLATORI N, BOYER JL, STRANGE K: Swelling-activated anion conductance in skate hepatocytes: Regulation by cell Cl , an ATP. *Am J Physiol (Cell Physiol)* (in press)
51. BALLATORI N, TRUONG AT, JACKSON PS, STRANGE K, BOYER JL: ATP depletion and inactivation of an ATP-sensitive taurine channel by classic ion channel blockers. *Mol Pharmacol* (in press)
52. POLLARD CE: A volume-sensitive Cl^- conductance in a mouse neuroblastoma *x* rat dorsal root ganglion cell line (F11). *Brain Res* 614:178–184, 1993
53. OIKE M, DROOGMANS G, NILIUS B: The volume-activated chloride current in human endothelial cells depends on intracellular ATP. *Pflügers Arch* 427:184–186, 1994
54. GULLANS SR, MANDEL LJ: Coupling of energy to transport in proximal and distal nephron, in *The Kidney: Physiology and Pathophysiology*, edited by DW SELDIN, G GIEBISCH, New York, Raven Press, Ltd., 1992, p 1291
55. EKHOLM A, KATSURA K, KRISTIAN T, LIU M, FOLBERGROVA J, SIESJO BK: Coupling of cellular energy state and ion homeostasis during recovery following brain ischemia. *Brain Res* 604:185–191, 1993
56. ELEFF SM, SCHLEIEN CL, KOEHLER RC, SHAFFNER DH, TSITLIK J, HALPERIN HR, ROGERS MC, TRAYSTMAN RJ: Brain bioenergetics during cardiopulmonary resuscitation in dogs. *Anesthesiology* 76:77–84, 1992

57. STRANGE K: Maintenance of cell volume in the central nervous system. *Pediatr Nephrol* 7:689–697, 1993
58. WRIGHT SH: Alanine and taurine transport by the gill epithelium of a marine bivalve: Effect of sodium on influx. *J Membr Biol* 95:37–45, 1987
59. HAWKINS AJS, HILBISH TJ: The costs of cell volume regulation: Protein metabolism during hyperosmotic adjustment. *J Mar Biol Assoc UK* 72:569–578, 1992
60. ULFENDAHL HR, WOLGAST M: Renal circulation and lymphatics, in *The Kidney: Physiology and Pathophysiology*, edited by DW SELDIN, G GIEBISCH, New York, Raven Press, Ltd., 1992, p 1017
61. MOTAIS R, GUIZOUARN H, GARCIA-ROMEU F: Red cell volume regulation: The pivotal role of ionic strength in controlling swelling-dependent transport systems. *Biochim Biophys Acta* 1075:169–180, 1991
62. STRANGE K: Are all cell volume changes the same? *News Physiol Sci* 9:223–228, 1994
63. MCMANUS M, SERHAN C, JACKSON PS, STRANGE K: Ketoconazole blocks organic osmolyte efflux independent of its effect on arachidonic acid conversion. *Am J Physiol (Cell Physiol)* 267:C266–C271, 1994
64. THIEMANN A, GRUNDER S, PUSCH M, JENTSCH TJ: A chloride channel widely expressed in epithelial and non-epithelial cells. *Nature* 356:57–60, 1992
65. GRUNDER S, THIEMANN A, PUSCH M, JENTSCH TJ: Regions involved in the opening of CIC-2 chloride channel by voltage and cell volume. *Nature* 360:759–762, 1992
66. JALONEN T: Single-channel characteristics of the large-conductance anion channel in rat cortical astrocytes in primary culture. *Glia* 9:227–237, 1993
67. SCHWIEBERT EM, MILLS JW, STANTON BA: Actin based cytoskeleton regulates a Cl^- channel and cell volume in renal cortical collecting duct cell line. *J Biol Chem* 269:7081–7089, 1994
68. FALKE LC, MISLER S: Activity of ion channels during volume regulation by clonal N1E115 neuroblastoma cells. *Proc Natl Acad Sci USA* 86:3919–3923, 1989
69. CHRISTENSEN O, HOFFMANN EK: Cell swelling activates K^+ and Cl^- channels as well as nonselective, stretch-activated cation channels in Ehrlich ascites tumor cells. *J Membr Biol* 129:13–36, 1992
70. CHRISTENSEN O, SIMON M, RANDLEV T: Anion channels in a leaky epithelium. A patch clamp study of the choroid plexus. *Pflügers Arch* 415:37–46, 1989
71. DIENER M, NOBLES M, RUMMEL W: Activation of basolateral Cl^- channels in the rat colonic epithelium during regulatory volume decrease. *Pflügers Arch* 421:530–538, 1992
72. GILL DR, HYDE SC, HIGGINS CF, VALVERDE MA, MINTENIG GM, SEPULVEDA FV: Separation of drug transport and chloride channel functions of the human multidrug resistance P-glycoprotein. *Cell* 71:23–32, 1992
73. VALVERDE MA, DIAZ M, SEPULVEDA FV: Volume-regulated chloride channels associated with the human multidrug-resistance P-glycoprotein. *Nature* 355:830–833, 1992
74. DIAZ M, VALVERDE MA, HIGGINS CF, RUCAREANU C, SEPULVEDA FV: Volume-activated chloride channels in HeLa cells are blocked by verapamil and dideoxyforskolin. *Pflügers Archv* 422:347–353, 1993
75. ALTENBERG GA, DEITMER JW, GLASS DC, REUSS L: P-glycoprotein-associated Cl^- currents are activated by cell swelling but do not contribute to cell volume regulation. *Cancer Res* 54:618–622, 1994
76. KROUSE ME, LUCKIE DB, HARPER KL, LAW TC, SIKIC BI, WINE JJ: MDR/P-glycoprotein expression facilitates swelling Cl^- current activation but is probably not the channel. *Pediatr Pulmonol* 17:631994
77. EHRING GR, OSIPCHUK YV, CAHALAN MD: Volume-activated chloride channels in drug-sensitive and resistant cell lines. (abstract) *Biophys J* 66:A142, 1994
78. OSIPCHUK YV, EHRING GR, CAHALAN MD: Pharmacological sensitivities of volume-activated chloride conductance and rhodamine 123 efflux in drug-sensitive and -resistant cell lines. (abstract) *Biophys J* 66:A142, 1994
79. MCEWAN GTA, HUNTER J, HIRST BH, SIMMONS NL: Volume-activated Cl^- secretion and transepithelial vinblastine secretion mediated by P-glycoprotein are not correlated in cultured human T84 intestinal epithelial layers. *FEBS Lett* 304:233–236, 1992
80. OKADA Y, PETERSEN CCH, KUBO M, MORISHIMA S, TOMINAGA M: Osmotic swelling activates intermediate-conductance Cl^- channels in human intestinal epithelial cells. *Jpn J Physiol* 44:403–409, 1994
81. JACKSON PS, STRANGE K: Single channel properties of a volume-sensitive anion conductance. Noise analysis reveals that current activation occurs by increases in channel number. *J Gen Physiol* 105:643–660, 1995
82. PAULMICHL M, LI Y, WICKMAN K, ACKERMAN M, PERALTA E, CLAPHAM D: New mammalian chloride channel identified by expression cloning. *Nature* 356:238–241, 1992
83. KRAPIVINSKY GB, ACKERMAN MJ, GORDON EA, KRAPIVINSKY LD, CLAPHAM DE: Molecular characterization of a swelling-induced chloride conductance regulatory protein, pICln. *Cell* 76:439–448, 1994
84. BENZ R: Structure and function of porins from gram-negative bacteria. *Ann Rev Microbiol* 42:359–393, 1988
85. BENZ R, BAUER K: Permeation of hydrophilic molecules through the outer membrane of gram-negative bacteria. Review on bacterial porins. *Eur J Biochem* 176:1–19, 1988
86. ROSENBUSCH JP: Structural and functional properties of porin channels in *E. coli* outer membranes *Experientia* 46:167–173, 1990
87. BERRIER C, COULOMBE A, HOUSSIN C, GHAZI, A: Voltage-dependent cationic channel of *Escherichia coli. J Membr Biol* 133:119–127, 1993
88. BERRIER C, COULOMBE A, HOUSSIN C, GHAZI A: Fast and slow kinetics of porin channels from *Esherichia coli* reconstituted into giant liposomes and studied by patch-clamp. *Fed Eur Biochem Soc* 306:251–256, 1992